European Journal of Pharmacology 835 (2018) 52–60

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Full length article

Sumatriptan effects on morphine-induced antinociceptive tolerance and physical dependence: The role of nitric oxide

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ARTICLE INFO

Keywords:
Morphine
Sumatriptan
Antinociception
Tolerance
Dependence
Nitric oxide

ABSTRACT

Sumatriptan, a 5HT (5-hydroxytryptamine)$_{1B/1D}$ receptor agonist, showed neuroprotection in different studies. The aim of the present study was to investigate the effect of sumatriptan on morphine-induced antinociceptive tolerance and physical dependence. We also investigated the possible role of nitric oxide (NO) on sumatriptan effects.

Tolerance was induced by morphine injection (50, 50, 75 mg/kg) three times daily for five days. Antinociceptive latency after acute and chronic treatment with sumatriptan (0.001, 0.01, 0.1 and 1 mg/kg) was measured by hot plate test in morphine-dependent animals. To investigate the possible involvement of NO, different isoforms of nitric oxide synthase (NOS) inhibitors including L-NAME, aminoguanidine and 7-nitroindazole were co-administered with sumatriptan. Nitrite level in mice hippocampus was quantified by Griess method. To examine the role of sumatriptan on physical dependence, three parameters of withdrawal signs were recorded after injection of naloxone (4 mg/kg).

Acute treatment with sumatriptan (0.01, 0.1 and 1 mg/kg) attenuated the antinociceptive tolerance ($P < 0.001$). Chronic injection of sumatriptan (0.001, 0.01 and 0.1 mg/kg), as well, decreased the antinociceptive tolerance ($P < 0.001$). Moreover, co-administration of NOS inhibitors prevented the effects of sumatriptan. Sumatriptan significantly increased the level of nitrite only after chronic administration. Sumatriptan administration showed no alteration in naloxone-precipitated withdrawal signs.

Acute and chronic administration of sumatriptan attenuated morphine antinociceptive tolerance; at least in chronic phase via nitrergic pathway. Our data did not support beneficial effects of sumatriptan on morphine-induced physical dependence in mice.

1. Introduction

The long-term administration of morphine and related opioids remains limited in pain management due to its adverse effects such as tolerance and dependence phenomena (Mao et al., 1995; Trujillo and Akil, 1991). Tolerance occurs when the efficacy of drug diminishes by continued usage; therefore, dose increasing is required to maintain the same therapeutic effect. Dependence consists of psychological and physical components. Psychological dependence is an obsessive need for seeking the drug while physical part develops when drug cessation causes withdrawal signs (Bläsig et al., 1973; Way et al., 1969).

To obtain a solution to prevent these two issues, it's essential to study the probable mechanisms involved in morphine-induced tolerance and dependence. Among all the studies investigated the

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https://doi.org/10.1016/j.ejphar.2018.07.021
Received 22 January 2018; Received in revised form 2 July 2018; Accepted 12 July 2018
Available online 06 August 2018

mechanisms contributed to opioid-induced tolerance and dependence, the majority of evidence verified the involvement of nitric oxide (NO) pathway (Herman et al., 1995; Mao, 1999; Marek et al., 1991; Nestler, 2004).

Sumatriptan, a selective $5HT_{1B/1D}$ receptor agonist, is a well-known drug for treatment of migraine and cluster headache. It's a well-tolerated drug with minor, rare and transient adverse effects (Dechant and Clissold, 1992; Ikeda et al., 2002). Although the mechanisms of action of sumatriptan need to be clarified, growing body of evidence presumed NO-dependent pathway as one of the probable mechanisms involved in its beneficial effects. Sumatriptan protective effects are mediated by inhibition of NO-induced calcium gene-related peptide (CGRP) synthesis, altering the balance of NO and superoxide in brain, and modulating the bioavailability of NO in central nervous system (CNS) (Dechant and Clissold, 1992; Ikeda et al., 2002; Stepień et al., 1999).

Nitric oxide, which is derived from amino acid L-arginine by the enzyme nitric oxide synthase (NOS), is an essential agent to produce cyclic guanosine monophosphate (cGMP). There are three identified isoforms of NOS, including inducible (iNOS), neuronal (nNOS) and endothelial (eNOS), expressed in different organs (Förstermann and Sessa, 2011). Nitric oxide plays an important role in numerous physiological and pathological conditions of CNS (Buisson et al., 1993; Montague et al., 1994; Szabó, 1996). A great body of evidence supported the involvement of NO/cGMP pathway on the morphine-induced tolerance and dependence. It has been shown that the suppression of NO by NOS inhibitors could suppress both the tolerance to morphine-induced antinociception and the withdrawal symptoms induced by naloxone (Babey et al., 1994; Elliott et al., 1994; Homayoun et al., 2003; Mao et al., 1995).

The purpose of our study was to test the hypothesis of sumatriptan effects on the morphine-induced antinociceptive tolerance and physical dependence in mice. We also examined the involvement of NO pathway in the possible effects of sumatriptan.

2. Materials and methods

2.1. Animals

The experiment was carried out on male NMRI mice (Naval Medical Research Institute), 6–7 weeks old, weighing 25–30 g. Animals were housed in cages under standard laboratory conditions (12-h light/dark cycle with an average temperature of 22 ± 2 °C and humidity of 55 ± 2%) with free access to food and tap water except for the time of experimental procedures. Each experimental group consists of 6–8 mice and each mouse was used once during the study. All the experiments were performed at the same time of every day. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) with the approval of Research and Medical Ethics Committees of Tehran University of Medical Science. The study consists of different experimental groups as follows: group 1: morphine alone, groups 2–5: acutely received four different doses of sumatriptan, groups 6–9: chronically received four different doses of sumatriptan, groups 10–13: morphine + four different acute doses of sumatriptan, groups 14–16: morphine + sumatriptan + three different isoforms of acute NOS inhibitors, groups 17–20: morphine + four different doses of chronic sumatriptan, groups 21–23: morphine + sumatriptan + three isoforms of chronic NOS inhibitors, groups 24–27: morphine + acute sumatriptan + naloxone, groups 28–31: morphine + chronic sumatriptan + naloxone.

2.2. Chemicals

The drugs that were used throughout this study were: sumatriptan, a $5HT_{1B/1D}$ receptor agonist; morphine sulfate, an opioid receptor agonist; L-NAME [L-NG-Nitro-L-arginine methyl ester hydrochloride], a non-specific inhibitor of NOS; aminoguanidine, a selective inhibitor of iNOS; 7-NI [7-nitroindazole], a selective inhibitor of nNOS; naloxone hydrochloride, an opioids receptor antagonist. Drugs were administered intraperitoneally (i.p.) in the volume of 10 ml/kg of mouse body weight. All drugs were dissolved in normal saline (NaCl 0.9%) freshly for use except for 7-NI which was dissolved in a 1% aqueous solution of dimethyl sulfoxide (DMSO), followed by sonication. Morphine sulfate was purchased from TEMAD, IRAN, naloxone was provided from Tolid Daru, Co Ltd, Tehran Iran, and all other drugs were purchased from Sigma, USA.

2.3. Induction of antinociceptive tolerance and dependence to morphine

The experiment was performed to assess two main problems of continued usage of morphine including tolerance and dependence. To induce antinociceptive tolerance, multiple injections of morphine were administered three times daily for 4 consecutive days with the doses of 50 mg/kg (8:00 a.m.), 50 mg/kg (11:00 a.m.) and 75 mg/kg (4:00 p.m.) (the third dose was higher in order to prevent withdrawal signs during night). On the last day of each experiment (5th day), animals received a single dose of morphine (50 mg/kg). The protocol for induction of antinociceptive tolerance to morphine was based on previous studies (Javadi et al., 2013). To assess antinociceptive threshold and degree of tolerance hot plate test was conducted.

The method of utilizing hot plate test for evaluating antinociceptive property was firstly described by Eddy and Leimbach (Eddy and Leimbach, 1953). The device consists of an electrically heated surface (50 ± 2 °C) covered with a plexiglass tube (18 cm high × 22 cm diameter) (Tahghigh-Gostaran-Teb, Iran). The antinociceptive threshold was defined as a time interval (s) between placing the animal on the heated surface and pain response (licking the hind paw or jumping with all four feet). Antinociceptive effect of morphine was assessed 60 min after the first injection of morphine on first, third and fifth days of the experiment. If the animals could not respond within 90 s, they were removed from the hot plate to prevent tissue damage. The increase in a time of animal response considered as antinociceptive induction and the decrease of antinociceptive threshold was determined as the degree of tolerance.

In aim of rendering physical dependence, morphine was injected three times daily with doses of 50 mg/kg (8:00 a.m.), 50 mg/kg (11:00 a.m.) and 75 mg/kg (4:00 p.m.) (the higher dose was administered to prevent withdrawal signs overnight) for 4 days and a single dose of 100 mg/kg on the last day of the study (fifth day).

To induce withdrawal signs, naloxone (4 mg/kg, i.p.) was administered one h after the last dose of morphine (100 mg/kg). After naloxone injection, each animal was placed in separated plexiglass cylinder (40 cm long, 25 cm wide and 45 cm high). Animals were observed for one h, and signs of withdrawal including jumping and rearing were recorded throughout this time. Percentage of weight loss as another sign of withdrawal was determined by measuring animal's weight before and 60 min after naloxone injection.

2.4. Assessing the effect of sumatriptan on morphine antinociceptive tolerance and naloxone-induced withdrawal signs

To investigate the antinociceptive property of sumatriptan, these doses of the drug were administrated alone (acute and chronic with four doses of 0.001, 0.01, 0.1 and 1 mg/kg) without morphine injection 45 min before hot plate test.

We evaluated the effect of sumatriptan on morphine-induced antinociceptive tolerance based on two protocols: In the first protocol, different doses of sumatriptan (0.001, 0.01, 0.1 and 1 mg/kg) were injected 45 min only before the last dose of morphine on the last day of the experiment in order to evaluate the acute treatment with sumatriptan on morphine antinociceptive tolerance.

In the second protocol, four different doses of sumatriptan (0.001,

0.01, 0.1 and 1 mg/kg) were administered 45 min prior to injection of every dose of morphine three times daily for 5 days. To assess the effect of sumatriptan on antinociceptive tolerance hot plate test was performed on first, third and fifth days of the study.

In order to determine the role of sumatriptan in morphine physical dependence and naloxone-induced withdrawal signs, various doses of sumatriptan (0.001, 0.01, 0.1 and 1 mg/kg) were administered in two different protocols similar to the method that was described previously for the effect of sumatriptan on antinociceptive tolerance.

To evaluate the effect of acute and chronic doses of sumatriptan on morphine dependence, naloxone precipitated withdrawal manifestations including jumping, rearing, and percentage of weight loss were determined 45 min after injection of morphine and immediately after naloxone injection on the last day.

2.5. Assessing the antinociceptive latency in the sumatriptan-treated group after the intervention of NOS inhibitors administration

To assess the role of NO on effects of acute treatment with sumatriptan, a single dose of 7-NI (40 mg/kg), L-NAME (5 mg/kg) and aminoguanidine (100 mg/kg) were administered only on the fifth day 45 min before the single injection of sumatriptan. To determine the role of NO pathway on modulating effect of chronic injection of sumatriptan, NOS inhibitors including 7-NI (15 mg/kg), L-NAME (2 mg/kg), aminoguanidine (50 mg/kg) were administered 45 min before every dose of sumatriptan (90 min before morphine administration) during five days of experiment. Hot plate test was performed 45 min after morphine administration both in acute and chronic phases. We selected sub-effective doses of NOS inhibitors based on our previous study (Hassanipour et al., 2016b).

2.6. Nitrite level assay in mice hippocampus

Rapid oxidation of NO leads to nitrite production. Mice of each study group were selected to measure nitrite in their hippocampus. Nitrite level was assessed by Griess reaction (Tsikas, 2007), briefly, frozen hippocampi were homogenized by addition of lysis buffer solution (pH = 8, with an amount of 4–6 times of tissue sample weight), homogenized samples then incubated at room temperature (20 ± 10 °C) for ten min followed by centrifugation (13,400 RCF) for 15 min. Supernatants obtained from centrifugation were assessed for nitrite level assay.

2.7. Data analysis

The results are expressed as mean ± standard error of the mean (S.E.M.). To perform the analysis, GraphPad Prism data analysis program version 6 was used (GraphPad Software San Diego, CA, USA). Differences within experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons and two-way ANOVA followed by Bonferroni post hoc test. P value < 0.05 was considered significant for all analyses.

3. Results

The summarized result of all behavioral studies is presented in Table 1.

3.1. Induction of morphine antinociceptive tolerance

Fig. 1 shows the antinociceptive tolerance to morphine. Administration of morphine during 5 days (three times daily for 4 consecutive days with the doses of 50 mg/kg, 50 mg/kg, and 75 mg/kg; and a single dose of 50 mg/kg on 5th day) leads to the development of tolerance which was assessed by hot plate test. The result revealed that antinociceptive latency on the fifth day is significantly lower than the latencies of the first and third days and near to the antinociceptive threshold of the control group ($P < 0.001$).

3.2. Assessment of the antinociceptive effect of sumatriptan

Antinociceptive latency after both acute and chronic administration of sumatriptan at doses of 0.001, 0.01, 0.1 and 1 mg/kg was compared to the control group which only received saline. As illustrated in the Fig. 2 antinociception threshold after acute and chronic injection of sumatriptan did not alter significantly in comparison with the control group but it was significantly lower than the antinociceptive latency in morphine-treated animals ($P < 0.001$).

3.3. Evaluation of the effect of acute administration of sumatriptan on morphine-induced antinociceptive tolerance

The effect of acute administration of sumatriptan on morphine-induced antinociceptive tolerance was assessed at different doses (0.001, 0.01, 0.1 and 1 mg/kg). The results showed that sumatriptan with doses of 0.01, 0.1 and 1 mg/kg markedly increased the antinociceptive threshold compared to morphine-treated group ($P < 0.001$; Fig. 3). However, the acute administration of sumatriptan (0.001 mg/kg) did not alter the morphine-induced antinociceptive tolerance in mice.

3.4. Role of NOS inhibitors in the effects of acute administration of sumatriptan on the morphine-induced antinociceptive tolerance

Fig. 4 shows the modulating role of NO on the effects of acute treatment with sumatriptan on morphine-induced antinociceptive tolerance. NOS inhibitors including L-NAME, a non-specific NOS inhibitor (5 mg/kg), 7-NI, a specific nNOS inhibitor (40 mg/kg), and aminoguanidine, a specific iNOS inhibitor (100 mg/kg), were administered 45 min before sumatriptan injection. Co-treatment with all types of NOS inhibitors reversed the effect of sumatriptan on the antinociceptive tolerance. As demonstrated in Fig. 4, antinociceptive latency has been alleviated significantly via administration of NOS inhibitors in comparison to the sumatriptan plus morphine-treated group which did not receive NOS inhibitors.

3.5. Effect of chronic administration of sumatriptan on morphine antinociceptive tolerance

As shown in Fig. 5, it is revealed that pain threshold in animals which were chronically treated with morphine and sumatriptan (0.1 and 0.001 mg/kg) was enhanced significantly in comparison with morphine-treated group ($P < 0.001$) on the last day of the experiment. Moreover, chronic administration of sumatriptan at 0.01 mg/kg inhibited the antinociceptive tolerance effect compared to morphine group ($P < 0.01$) on the fifth day. Co-treatment of morphine and sumatriptan at 1 mg/kg showed no significant effect on antinociceptive tolerance in comparison with animals which were treated only with morphine.

3.6. Effect of NOS inhibitors in chronic administration of sumatriptan on the antinociceptive tolerance induced by morphine

Fig. 6 illustrates the involvement of NO on the effect of sumatriptan on morphine-induced antinociceptive tolerance. Three of NOS inhibitors including L-NAME (2 mg/kg), 7-NI (15 mg/kg) and aminoguanidine (50 mg/kg) were injected 45 min before every injection of sumatriptan (0.1 mg/kg) for five days. The results revealed that 7-NI significantly decreased the antinociceptive latency in comparison to the sumatriptan-treated group ($P < 0.01$). Moreover, as it has been shown in Fig. 6, chronic co-administration of sumatriptan with L-NAME and aminoguanidine completely blocked the effect of sumatriptan ($P < 0.05$).

Table 1
The effects of sumatriptan (SUMA) administration on morphine (MOR)-induced antinociceptive tolerance and physical dependence. In expression phase sumatriptan was injected only at the last day of experiment (5th day) and in induction phase sumatriptan was co-administered with morphine three times daily for 5 days. The role of nitric oxide (NO) was investigated by administration of NO modulators (L-NAME, aminoguanidine, and 7-NI) in both induction and expression phases. n: number of mice in each experimental group.

Experiments	Drugs				
	Saline	**MOR**	**SUMA (0.001, 0.01, 0.1, 1 mg/kg)**	**MOR + SUMA (0.001, 0.01, 0.1, 1 mg/kg)**	**MOR + SUMA 0.1 mg/kg + NO modulators**
Tolerance (expression)	**No effect** n = 8	↓**Antinociception** n = 8	**No effect** Each group n = 8	↑**Antinociception** (0.01,0.1, and 1 mg/kg SUMA) Each group n = 8	↓**Antinociception** n = 8
Tolerance (induction)	**No effect** n = 8	↓**Antinociception** n = 8	**No effect** Each group n = 8	↑**Antinociception** (0.01,0.1, and 1 mg/kg SUMA) Each group n = 8	↓**Antinociception** Each group n = 8
Dependence (expression)	**No effect** n = 8	↑**Dependence** n = 8	–	**No effect** n = 8	–
Dependence (induction)	**No effect** n = 8	↑**Dependence** n = 8	–	**No effect** n = 8	–

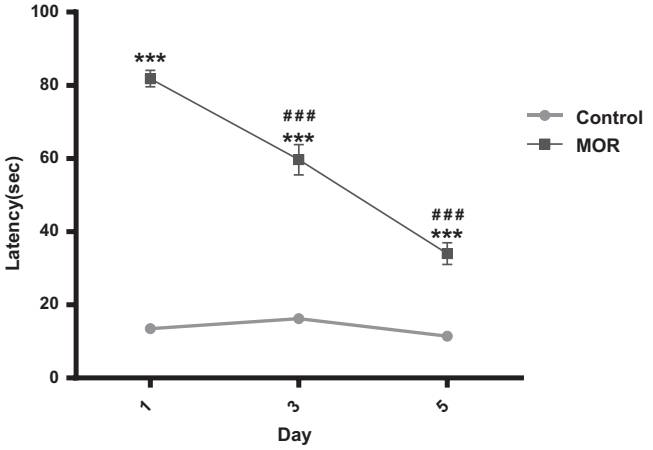

Fig. 1. Induction of antinociceptive tolerance to morphine (MOR). Morphine was administered three times daily for five consecutive days. Data are expressed as mean ± S.E.M. (n = 8). *** $P < 0.001$ compared to the control group. ### $P < 0.001$ compared to the antinociceptive latency of the first day of the experiment.

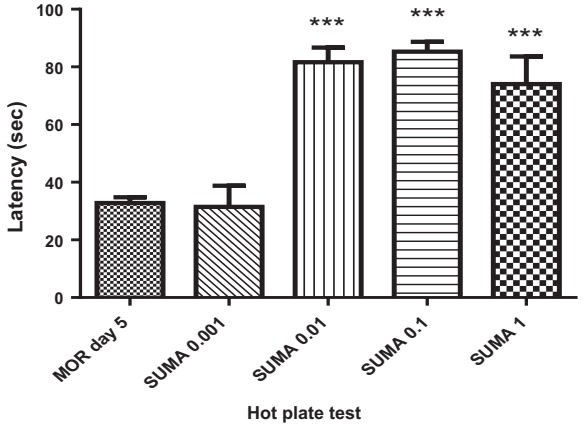

Fig. 3. The effect of acute injection of different doses of sumatriptan (SUMA) on morphine-induced antinociceptive tolerance. The sumatriptan doses were injected 45 min before the last dose of morphine (MOR) on the last day. Data are expressed as mean ± S.E.M. (n = 8). ***$P < 0.001$ compared to the morphine-treated group.

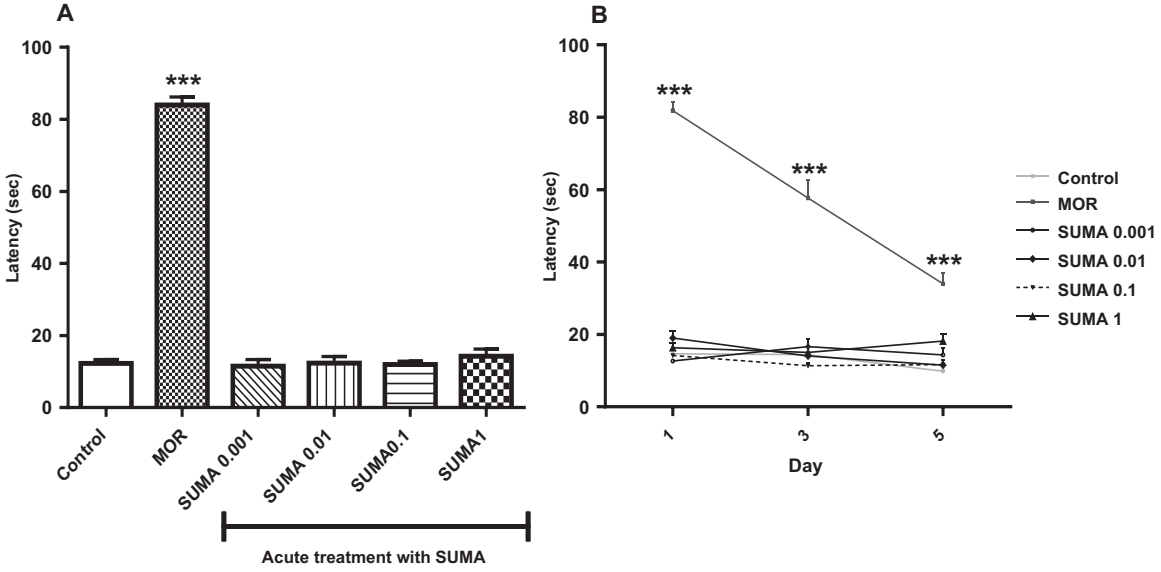

Fig. 2. Evaluation of the antinociceptive property of sumatriptan (SUMA) without co-administration of morphine (MOR). A. Acute injection of different doses of sumatriptan. B. Different doses of sumatriptan were administered three times daily for five days. Data are expressed as mean ± S.E.M. (n = 8). ***$P < 0.001$ compared to the control group.

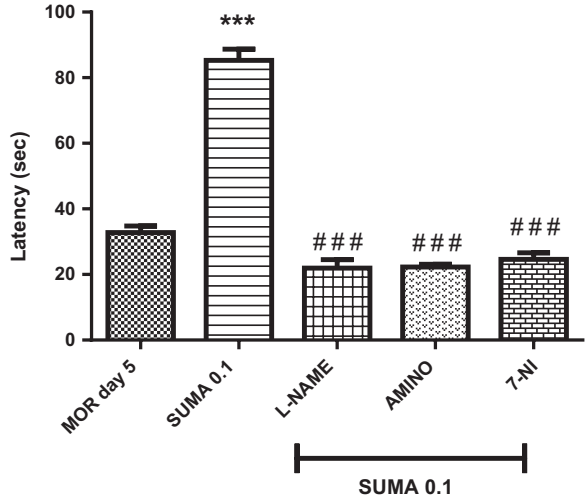

Fig. 4. The effect of acute administration of NOS inhibitors on the effects of sumatriptan (SUMA) in morphine (MOR)-induced antinociceptive tolerance. NOS inhibitors including L-NAME, a non-specific NOS inhibitor, 7-NI, a specific nNOS inhibitor, aminoguanidine (AMINO), a specific iNOS inhibitor, were injected 45 min before sumatriptan. ***P < 0.001 compared to morphine (MOR) on the fifth day. ### P < 0.001 compared to concomitant administration of sumatriptan with morphine. Data are expressed as mean ± S.E.M. (n = 8).

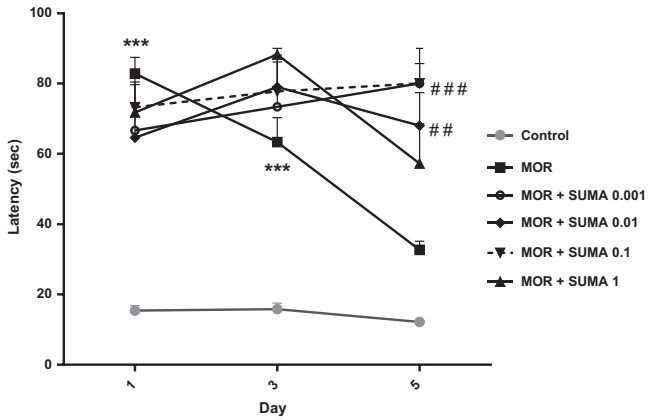

Fig. 5. The effect of chronic administration of sumatriptan (SUMA) on the antinociceptive tolerance to morphine (MOR). Different doses of sumatriptan were injected 45 min before every dose of morphine during five days. ***P < 0.001 compared to control group. ###P < 0.001 and ##P < 0.01 compared to morphine-treated group on the last day. Data are expressed as mean ± S.E.M. (n = 8).

3.7. Assessing the role of acute injection of sumatriptan on the naloxone-induced withdrawal signs and physical dependence to morphine

As illustrated in Fig. 7, naloxone injection in animals under chronic administration of morphine resulted in significant increase in withdrawal parameters including number of jumping, rearing and percentage of weight loss in comparison with saline-treated animals. Measuring the withdrawal parameters, as the degree of physical dependence, after injection of naloxone (4 mg/kg) did not reveal any inhibitory effect for acute sumatriptan-treated animals (all animals were dependent to morphine by chronic administration of morphine during five days). On the other hand, this data showed that physical dependence induced with naloxone injection was potentiated in the animals which received sumatriptan (1 mg/kg). Sumatriptan at 1 mg/kg increased the number of jumping, rearing and weight loss compared to the group that only received morphine (P < 0.05). Acute injection of sumatriptan at 0.01 mg/kg had a worsening effect on the number of rearing in morphine-dependent mice (P < 0.05).

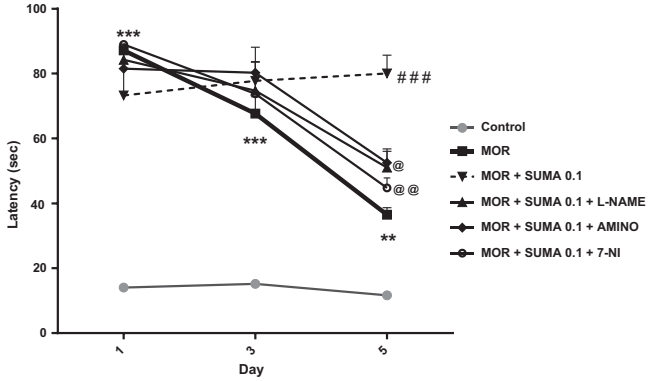

Fig. 6. The effect of chronic administration of NOS inhibitors on the effects of sumatriptan (SUMA) in morphine (MOR)-induced antinociceptive tolerance. L-NAME (2 mg/kg), aminoguanidine (AMINO; 50 mg/kg), and 7-NI (15 mg/kg) were injected 45 min before each dose of sumatriptan. Data are expressed as mean ± S.E.M. (n = 8). **P < 0.01 and ***P < 0.001 compared to control group. ###P < 0.001 in comparison to the morphine-treated group. @ P < 0.05 and @@P < 0.01 compared to the co-administration of SUMA 0.1 mg/kg + MOR.

3.8. Evaluating the chronic administration of sumatriptan on the morphine-induced dependence

Fig. 8 showed that chronic administration of sumatriptan did not protect animals from the withdrawal signs evoked by naloxone injection in morphine-dependent mice. Behavioral signs of morphine withdrawal like jumping, rearing and the percentage of weight loss showed no statistically significant difference in animals which were treated with sumatriptan and morphine compared to the group that only received morphine.

3.9. Measurement of the nitrite level

The results of nitrite level assay in mice hippocampi is depicted in Fig. 9. Chronic treatment with sumatriptan with doses of 0.001, 0.01 and 0.1 mg/kg significantly increased the level of nitrite compared to morphine- and saline-treated groups (P < 0.001). Alteration in the nitrite level at 1 mg/kg was not statistically significant.

Although the chronic administration of sumatriptan increased the nitrite level in mice hippocampi, acute treatment with sumatriptan showed no difference in the quantification of nitrite level compared to the morphine-treated group.

4. Discussion

The results of the present study revealed that acute and chronic administration of sumatriptan, a specific $5HT_{1B/1D}$ receptor agonist, could significantly alleviate morphine-induced antinociceptive tolerance. To obtain a comprehensive understanding of the role of NO pathway, NOS inhibitors were co-administered with sumatriptan; the results showed that inhibition of NO synthase blocks the sumatriptan effects on antinociceptive tolerance in both acute and chronic administration of sumatriptan in morphine-dependent mice. Acute and chronic treatment with sumatriptan did not show protective effect against withdrawal signs precipitated by naloxone challenge in morphine-dependent animals. As there was no conclusive data about the effect of sumatriptan on the morphine dependence, we did not use NOS inhibitors with sumatriptan in the section of dependence evaluation. Furthermore, the assessment of the nitrite level, as an end product of NO, in mice hippocampi revealed that the amount of NO increased in chronic sumatriptan-treated mice, but no difference was observed in the

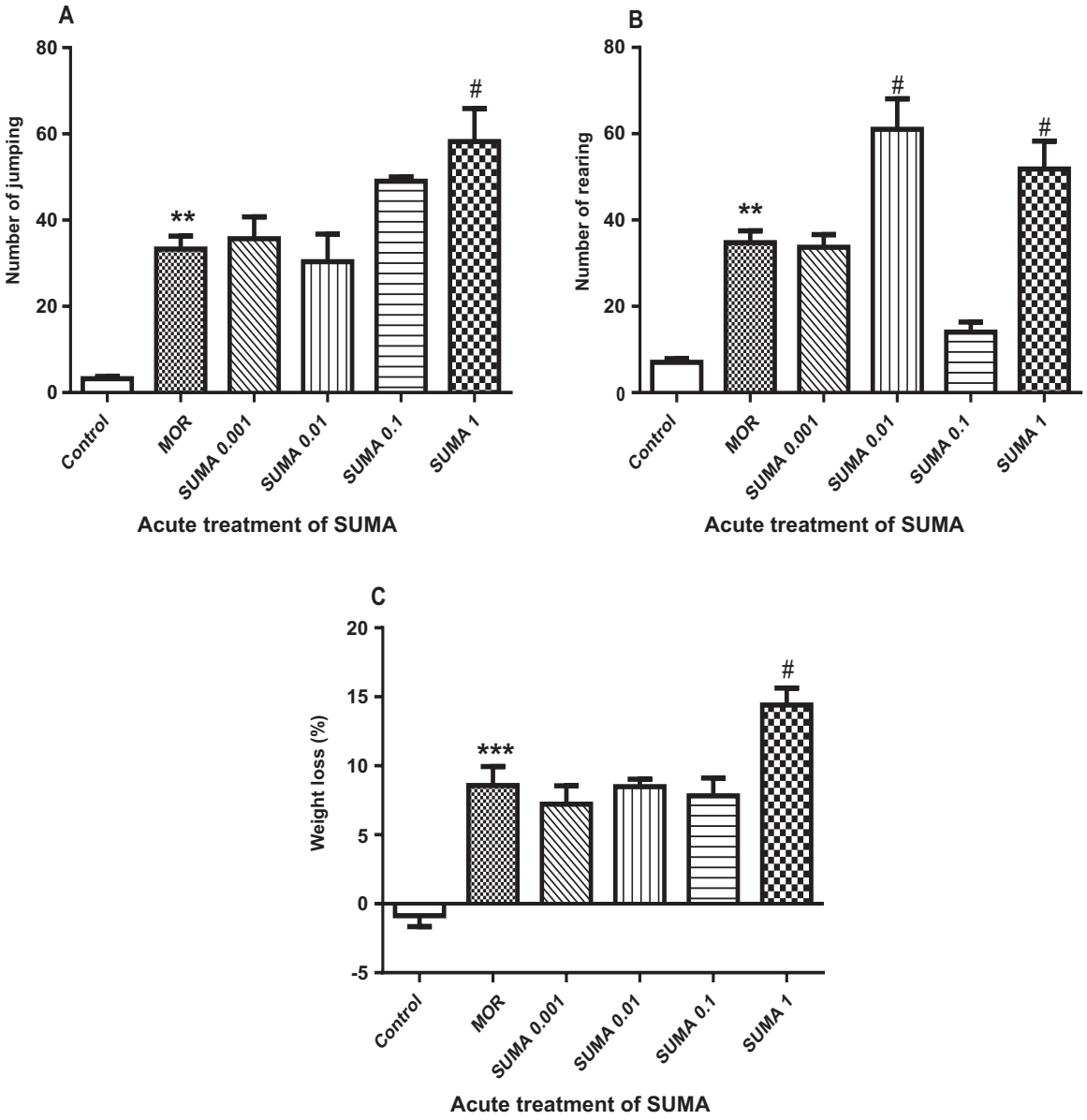

Fig. 7. The effect of acute treatment with sumatriptan (SUMA) on morphine (MOR)-induced physical dependence. Animals received different doses of sumatriptan 45 min before the last injection of morphine (100 mg/kg). Signs of drug cessation including A. number of jumping, B. number of rearing, and C. percentage of weight loss were measured after naloxone injection (4 mg/kg). Data are expressed as mean ± S.E.M. (n = 8). **P < 0.05 and ***P < 0.001 compared to control group. #P < 0.05 compared to morphine-treated group.

level of nitrite in acute treatment with sumatriptan.

Morphine and other opioids are considered standard antinociceptive treatment for decades (Mao et al., 1995). But prolonged exposure to these drugs is limited due to the rapid development of tolerance and dependence. Numerous molecular mechanisms have been suggested for these undesirable effects of morphine, including desensitization of μ-opioid receptor via receptor internalization, uncoupling from G-protein, up-regulation of cAMP pathway, activation of N-methyl D-aspartate (NMDA) system, central neuroimmune activation and NOS activation (Bailey and Connor, 2005; Ben-Eliyahu et al., 1992; Bian et al., 2012; Dang and Christie, 2012; Deleo et al., 2004; DuPen et al., 2007; Koch and Höllt, 2008; Kolesnikov et al., 1993; Koppert, 2007; Martini and Whistler, 2007; Ossipov et al., 2004). A great body of evidence suggests the NO pathway as a proven mechanism involved in morphine-induced tolerance/dependence phenomena. Studies showed that NO has a dual role in modulating the antinociceptive tolerance to morphine. For example, co-administration of L-arginine, the precursor of NO accelerated the morphine tolerance in some studies (Babey et al., 1994). The study that has been done on the mechanism involved in morphine antinociceptive effect by Dambisya and Lee (1996) revealed that NO has an important role in morphine tolerance/dependence phases and suggested that increase in the level of NO attenuates morphine-induced antinociceptive tolerance and physical dependence, while inhibition of NOS accelerated these two phenomena. Another study performed by Thorat et al. (1993) reported that inhibition of NOS might prevent the development of morphine antinociceptive tolerance. Although there is evidence of both preventive and exacerbating effects for NO pathway in previous studies, our findings on the role of NO in the development of tolerance to morphine were in favor of studies which suggested that increasing the level of NO has a protective effect against morphine-induced antinociceptive tolerance.

Critical role of opioids in pain management in clinical setting led to performing many investigations on the efficacy of different agents to reduce or prevent tolerance and dependence induced by morphine

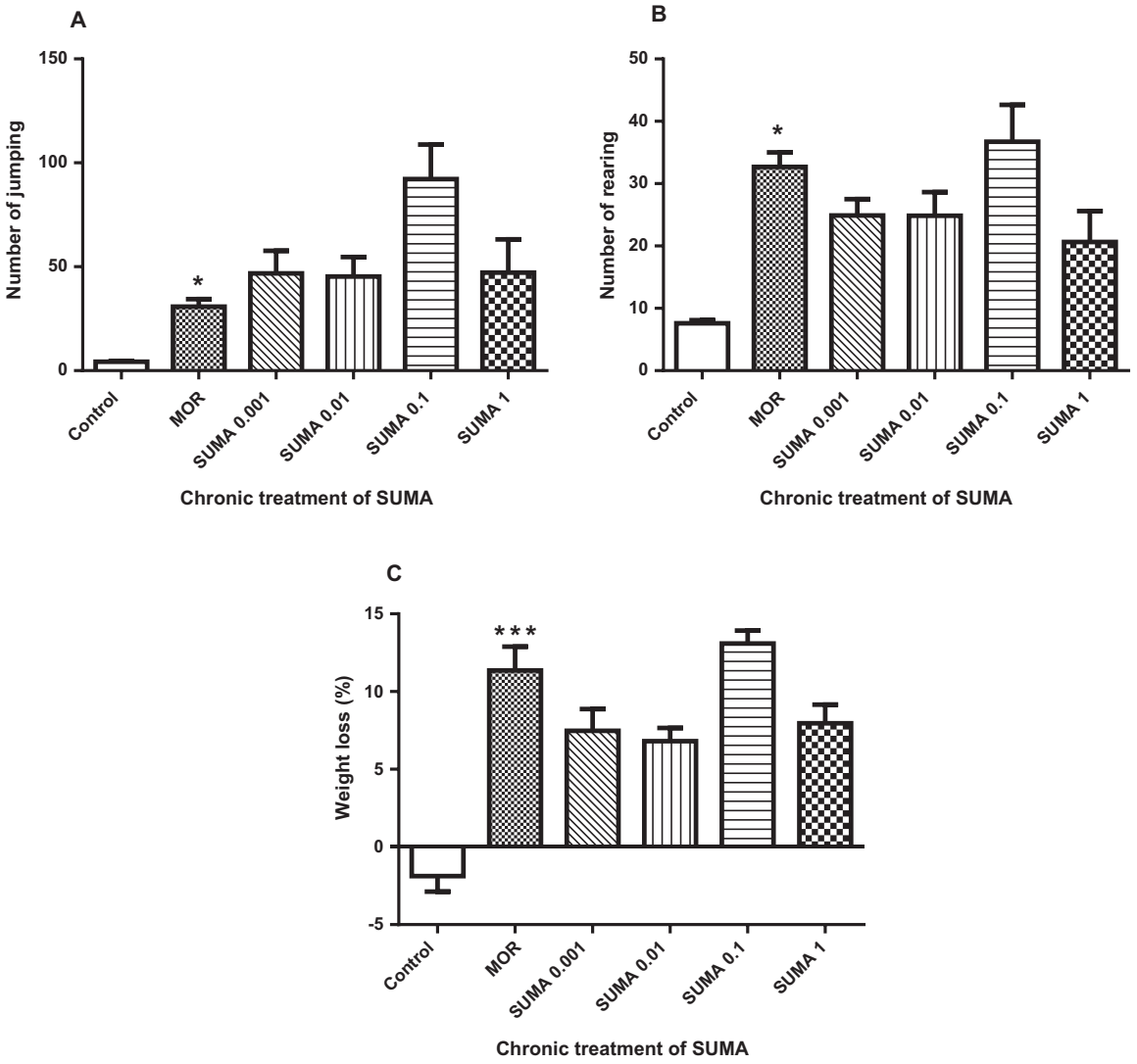

Fig. 8. The results of the chronic treatment of sumatriptan (SUMA) on the physical dependence to morphine (MOR). Animals were treated with different doses of sumatriptan 45 min before each dose of morphine three times daily for five days. A. number of jumping, B. number of rearing, and C. percentage of weight loss as three parameters of withdrawal signs were measured after naloxone injection (4 mg/kg). Data are expressed as mean ± S.E.M. (n = 8). *P < 0.05 and ***P < 0.001 compared to control group.

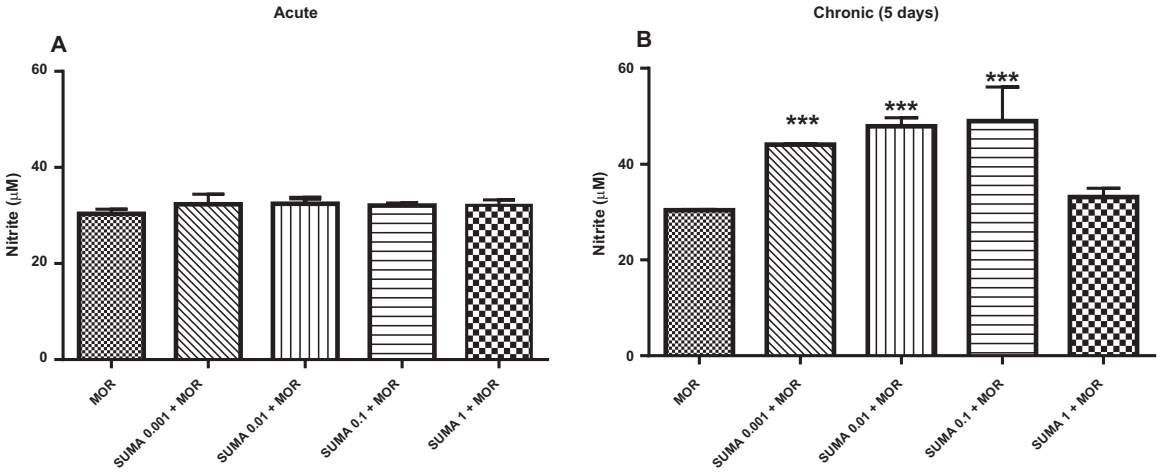

Fig. 9. The effect of the administration of sumatriptan (SUMA) on the nitrite level of mice hippocampi. A. Animals received a single injection of sumatriptan 45 min before the last dose of morphine (MOR). B. Sumatriptan was injected 45 min before daily treatment of morphine three times a day for 5 days. Data are expressed as mean ± S.E.M. (n = 8). **P < 0.01 and ***P < 0.001 compared to control group. #P < 0.05 and ##P < 0.01 compared to morphine-treated group.

(Hassanipour et al., 2016a; Mao et al., 1996).

Sumatriptan, a $5HT_{1B/1D}$ receptor agonist, is commonly known for managing migraine type headaches and their associated symptoms. Recent studies revealed further protective effects of sumatriptan besides its migraine pain relief property. Previous study suggested that sumatriptan has a neuroprotective effect in focal cerebral ischemia via improvement of brain blood flow in rats (Mies, 1998). Another investigation showed that chronic administration of sumatriptan improves depression and obsessive-compulsive disorder (OCD) in drug-resistant patients (Stern et al., 1998). Despite the previous studies about the effect of sumatriptan in migraine pain, we observed that sumatriptan with administrated doses in the current study did not exert antinociceptive property by itself (without morphine administration) in mice. Interestingly, sumatriptan raised the latency time which was decreased following 5-days treatment with morphine, that is, sumatriptan prevents morphine-induced antinociceptive tolerance.

Anti-migraine properties of sumatriptan are due to multiple mechanisms including direct vasoconstriction of the smooth muscles of dilated meningeal vessels, inhibition of the releasing the nociceptive neurotransmitters, increasing cholinergic neurotransmission, inhibition of the secretion of calcium gene-related peptide (CGRP) and modulation of NO signaling pathway (Dechant and Clissold, 1992; Durham and Russo, 1999; Ghelardini et al., 1997; Read and Parsons, 2000; Tepper et al., 2002). A large body of evidence implicated NO pathway as a major mechanism involved in pharmacological properties of sumatriptan (Akerman et al., 2002; Stepień et al., 1999; Tepper et al., 2002). In this study administration of NOS inhibitors including L-NAME (non-specific NOS inhibitor), 7-nitroindazole (specific neuronal NOS inhibitor), and aminoguanidine (specific inducible NOS inhibitor) inhibited the effect of acute and chronic administration of sumatriptan on the antinociceptive tolerance. In addition, chronic treatment with sumatriptan enhanced the nitrite level in mice hippocampi, but the acute administration did not exhibit this pattern. We assume that the inhibitory effect of acute administration of sumatriptan on antinociceptive tolerance is due to other mechanisms or involvement of other parts of brain, besides hippocampus, such as locus coeruleus, prefrontal cortex and glial cells (Ammon et al., 2003; Andrade et al., 1983; Rossetio et al., 1993; Song and Zhao, 2001).

We examined the effectiveness of treatment with sumatriptan on morphine physical dependence. The results revealed that acute administration of sumatriptan could not attenuate naloxone-precipitated opioids withdrawal signs. Furthermore, chronic treatment with sumatriptan did not modify the parameters of withdrawal signs evoked by naloxone challenge in morphine-dependent mice. Based on these data, sumatriptan could not prevent or attenuate physical dependence caused by prolonged exposure to opioids. This interesting finding indicated the dissociation between morphine-induced tolerance and physical dependence mechanisms. Pharmacological dissociation between tolerance to and dependence on morphine has been illustrated in previous studies. Aley and Levine (1997) demonstrated that administration of L-NAME blocks the antinociceptive tolerance induced by repeated administration of μ-opioid agonist enkephalin but does not prevent the development of dependence. Similarly, Gabra et al. (2008) demonstrated a significant decrease in morphine-induced antinociceptive tolerance via protein kinase C (PKC) and protein kinase A (PKA) inhibitors pretreatment, but these agents failed to prevent the development of physical dependence.

More investigations are highly recommended in order to clarify the role of sumatriptan, the exact molecular basis of these effects on antinociceptive tolerance and dependence, gene expression and protein expression in different brain areas, and investigating the possible implication of sumatriptan in clinical setting.

5. Conclusion

Our study concluded that sumatriptan could attenuate antinociceptive tolerance induced by chronic administration of morphine. This prevention was not observed in dependence phase. The results suggested that the role of sumatriptan on morphine effects is modulated at least in part via NO-dependent pathway.

Acknowledgments

The study was supported by a grant from Experimental Medicine Research Center, Tehran University of Medical Sciences (grant number 96-03-30-36167).

This work was supported by a grant (grants number 96002757) from Iran National Science Foundation (INSF).

Declarations of interest

None.

References

Akerman, S., Williamson, D.J., Kaube, H., Goadsby, P.J., 2002. The effect of anti-migraine compounds on nitric oxide-induced dilation of dural meningeal vessels. Eur. J. Pharmacol. 452, 223–228.

Aley, K., Levine, J., 1997. Dissociation of tolerance and dependence for opioid peripheral antinociception in rats. J. Neurosci. 17, 3907–3912.

Ammon, S., Mayer, P., Riechert, U., Tischmeyer, H., Höllt, V., 2003. Microarray analysis of genes expressed in the frontal cortex of rats chronically treated with morphine and after naloxone precipitated withdrawal. Brain Res. Mol. Brain Res. 112, 113–125.

Andrade, R., Vandermaelen, C.P., Aghajanian, G.K., 1983. Morphine tolerance and dependence in the locus coeruleus: single cell studies in brain slices. Eur. J. Pharmacol. 91, 161–169.

Babey, A.M., Kolesnikov, Y., Cheng, J., Inturrisi, C.E., Trifilletti, R.R., Pasternak, G.W., 1994. Nitric oxide and opioid tolerance. Neuropharmacology 33, 1463–1470.

Bailey, C.P., Connor, M., 2005. Opioids: cellular mechanisms of tolerance and physical dependence. Curr. Opin. Pharmacol. 5, 60–68.

Ben-Eliyahu, S., Marek, P., Vaccarino, A.L., Mogil, J.S., Sternberg, W.F., Liebeskind, J.C., 1992. The NMDA receptor antagonist MK-801 prevents long-lasting non-associative morphine tolerance in the rat. Brain Res. 575, 304–308.

Bian, J.-M., Wu, N., Su, R.-B., Li, J., 2012. Opioid receptor trafficking and signaling: what happens after opioid receptor activation? Cell. Mol. Neurobiol. 32, 167–184.

Bläsig, J., Herz, A., Reinhold, K., Zieglgänsberger, S., 1973. Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated withdrawal syndrome in rats. Psychopharmacology 33, 19–38.

Buisson, A., Lakhmeche, N., Verrecchia, C., Plotkine, M., Boulu, R.G., 1993. Nitric oxide: an endogenous anticonvulsant substance. NeuroReport 4, 444–446.

Dambisya, Y.M., Lee, T.L., 1996. Role of nitric oxide in the induction and expression of morphine tolerance and dependence in mice. Br. J. Pharmacol. 117, 914–918.

Dang, V.C., Christie, M.J., 2012. Mechanisms of rapid opioid receptor desensitization, resensitization and tolerance in brain neurons. Br. J. Pharmacol. 165, 1704–1716.

Dechant, K.L., Clissold, S.P., 1992. Sumatriptan. Drugs 43, 776–798.

Deleo, J.A., Tanga, F.Y., Tawfik, V.L., 2004. Neuroimmune activation and neuroinflammation in chronic pain and opioid tolerance/hyperalgesia. Neuroscientist 10, 40–52.

DuPen, A., Shen, D., Ersek, M., 2007. Mechanisms of opioid-induced tolerance and hyperalgesia. Pain Manag. Nurs. 8, 113–121.

Durham, P.L., Russo, A.F., 1999. Regulation of calcitonin gene-related peptide secretion by a serotonergic antimigraine drug. J. Neurosci. 19, 3423–3429.

Eddy, N.B., Leimbach, D., 1953. Synthetic analgesics. II. Dithienylbutenyl-and dithienylbutylamines. J. Pharmacol. Exp. Ther. 107, 385–393.

Elliott, K., Minami, N., Kolesnikov, Y.A., Pasternak, G.W., Inturrisi, C.E., 1994. The NMDA receptor antagonists, LY274614 and MK-801, and the nitric oxide synthase inhibitor, NG-nitro-L-arginine, attenuate analgesic tolerance to the mu-opioid morphine but not to kappa opioids. Pain 56, 69–75.

Förstermann, U., Sessa, W.C., 2011. Nitric oxide synthases: regulation and function. Eur. Heart J. 33, 829–837.

Gabra, B.H., Bailey, C.P., Kelly, E., Smith, F.L., Henderson, G., Dewey, W.L., 2008. Pretreatment with a PKC or PKA inhibitor prevents the development of morphine tolerance but not physical dependence in mice. Brain Res. 1217, 70–77.

Ghelardini, C., Galeotti, N., Nicolodi, M., Donaldson, S., Sicuteri, F., Bartolini, A., 1997. Involvement of central cholinergic system in antinociception induced by sumatriptan in mouse. Int. J. Clin. Pharmacol. Res. 17, 105–109.

Hassanipour, M., Amini-Khoei, H., Shafaroodi, H., Shirzadian, A., Rahimi, N., Imran-Khan, M., Rezayat, S.-M., Dehpour, A., 2016a. Atorvastatin attenuates the antinociceptive tolerance of morphine via nitric oxide dependent pathway in male mice. Brain Res. Bull. 125, 173–180.

Hassanipour, M., Shirzadian, A., Boojar, M.M.-A., Abkhoo, A., Abkhoo, A., Delazar, S., Amiri, S., Rahimi, N., Ostadhadi, S., Dehpour, A.-R., 2016b. Possible involvement of nitrergic and opioidergic systems in the modulatory effect of acute chloroquine treatment on pentylenetetrazol induced convulsions in mice. Brain Res. Bull. 121, 124–130.

Herman, B.H., Vocci, F., Bridge, P., 1995. The effects of NMDA receptor antagonists and

nitric oxide synthase inhibitors on opioid tolerance and withdrawal: medication development issues for opiate addiction. Neuropsychopharmacology 13, 269–293.
Homayoun, H., Khavandgar, S., Mehr, S.E., Namiranian, K., Dehpour, A., 2003. The effects of FK506 on the development and expression of morphine tolerance and dependence in mice. Behav. Pharmacol. 14, 121–127.
Ikeda, Y., Jimbo, H., Shimazu, M., Satoh, K., 2002. Sumatriptan scavenges superoxide, hydroxyl, and nitric oxide radicals: in vitro electron spin resonance study. Headache 42, 888–892.
Javadi, S., Ejtemaeimehr, S., Keyvanfar, H.R., Moghaddas, P., Aminian, A., Rajabzadeh, A., Mani, A.R., Dehpour, A.R., 2013. Pioglitazone potentiates development of morphine-dependence in mice: possible role of NO/cGMP pathway. Brain Res. 1510, 22–37.
Koch, T., Höllt, V., 2008. Role of receptor internalization in opioid tolerance and dependence. Pharmacol. Ther. 117, 199–206.
Kolesnikov, Y.A., Pick, C.G., Ciszewska, G., Pasternak, G.W., 1993. Blockade of tolerance to morphine but not to kappa opioids by a nitric oxide synthase inhibitor. Proc. Natl. Acad. Sci. USA 90, 5162–5166.
Koppert, W., 2007. Opioid-induced hyperalgesia – Pathophysiology and clinical relevance. Anaesthesist 9, 21–34.
Mao, J., 1999. NMDA and opioid receptors: their interactions in antinociception, tolerance and neuroplasticity. Brain Res. Brain Res. Rev. 30, 289–304.
Mao, J., Price, D.D., Caruso, F.S., Mayer, D.J., 1996. Oral administration of dextromethorphan prevents the development of morphine tolerance and dependence in rats. Pain 67, 361–368.
Mao, J., Price, D.D., Mayer, D.J., 1995. Mechanisms of hyperalgesian and morphine tolerance: a current view of their possible interactions. Pain 62, 259–274.
Marek, P., Ben-Eliyahu, S., Gold, M., Liebeskind, J.C., 1991. Excitatory amino acid antagonists (kynurenic acid and MK-801) attenuate the development of morphine tolerance in the rat. Brain Res. 547, 81–88.
Martini, L., Whistler, J.L., 2007. The role of mu opioid receptor desensitization and endocytosis in morphine tolerance and dependence. Curr. Opin. Neurobiol. 17, 556–564.
Mies, G., 1998. Neuroprotective effect of sumatriptan, a 5-HT 1D receptor agonist, in focal cerebral ischemia of rat brain. J. Stroke Cerebrovasc. Dis. 7, 242–249.
Montague, P.R., Gancayco, C.D., Winn, M.J., Marchase, R.B., Friedlander, M.J., 1994. Role of NO production in NMDA receptor-mediated neurotransmitter release in cerebral cortex. Science 263, 973–976.
Nestler, E.J., 2004. Historical review: molecular and cellular mechanisms of opiate and cocaine addiction. Trends Pharmacol. Sci. 25, 210–218.
Ossipov, M.H., Lai, J., King, T., Vanderah, T.W., Malan, T.P., Hruby, V.J., Porreca, F., 2004. Antinociceptive and nociceptive actions of opioids. Dev. Neurobiol. 61, 126–148.
Read, S.J., Parsons, A.A., 2000. Sumatriptan modifies cortical free radical release during cortical spreading depression: a novel antimigraine action for sumatriptan? Brain Res. 870, 44–53.
Rossetio, Z.L., Longu, G., Mercuro, G., Gessa, G.L., 1993. Extraneuronal noradrenaline in the prefrontal cortex of morphine-dependent rats: tolerance and withdrawal mechanis. Brain Res. 609, 316–320.
Song, P., Zhao, Z.-Q., 2001. The involvement of glial cells in the development of morphine tolerance. Neurosci. Res. 39, 281–286.
Stepień, A., Chalimoniuk, M., Strosznajder, J., 1999. Serotonin 5HT1B/1D receptor agonists abolish NMDA receptor-evoked enhancement of nitric oxide synthase activity and cGMP concentration in brain cortex slices. Cephalalgia 19, 859–865.
Stern, L., Zohar, J., Cohen, R., Sasson, Y., 1998. Treatment of severe, drug resistant obsessive compulsive disorder with the 5HT 1D agonist sumatriptan. Eur. Neuropsychopharmacol. 8, 325–328.
Szabó, C., 1996. Physiological and pathophysiological roles of nitric oxide in the central nervous system. Brain Res. Bull. 41, 131–141.
Tepper, S.J., Rapoport, A.M., Sheftell, F.D., 2002. Mechanisms of action of the 5-HT1B/1D receptor agonists. Arch. Neurol. 59, 1084–1088.
Thorat, S.N., Reddy, P.L., Bhargava, H.N., 1993. Evidence for the role of nitric oxide in κ-opiate tolerance in mice. Brain Res. 621, 171–174.
Trujillo, K.A., Akil, H., 1991. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 251, 85–88.
Tsikas, D., 2007. Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 851, 51–70.
Way, E.L., Lou, H.H., Shen, F.-H., 1969. Simultaneous quantitative assessment of morphine tolerance and physical dependence. J. Pharmacol. Exp. Ther. 167, 1–8.